

Paper III

Including Online Repository tables and figures not already included in the
Synopsis

Letter to the Editor

Cross-reactivity in fish allergy: A double-blind, placebo-controlled food-challenge trial

To the Editor:

Fish is a healthy nutrient and a common food allergen. Cross-reactivity between fish species exists, and some patients report tolerance to certain species¹ but data from food-challenge trials are scarce.^{2,3} Parvalbumin is the major fish allergen⁴⁻⁶ while recently fish muscle enolases and aldolases were identified as new fish allergens.⁷ We present the first double-blind, placebo-controlled food-challenge (DBPCFC) trial with different fish species, evaluating the correlation between clinical reactivity and IgE reactivity to fish-allergen molecules in fish-allergic patients.

In this trial, 35 subjects (5-19 years) with physician-diagnosed fish allergy and sensitization to fish allergen underwent DBPCFC with cod, salmon, and mackerel, followed by open food challenges (OFCs) if DBPCFCs were negative. Study design (Fig E1), patients' background characteristics (Table E1), and methods are described in this article's Online Repository at www.jacionline.org.

Any allergic symptoms were observed/reported from 33 (cod), 28 (salmon), and 28 (mackerel) participants. Five participants reported mild, transient subjective symptoms on placebo challenge but had unequivocal findings during the DBPCFC-active arm and are thus included in the analysis (see Table E2 in this article's Online Repository at www.jacionline.org).

We found tolerance to at least 1 of 3 fish species (partially tolerant) in 10 (29%) participants regarding any symptoms (Table I). Table II presents reactivity in partially tolerant participants. There was no difference between nontolerant (reacting to all 3 fish species) and partially tolerant participants regarding age, sex, other allergies, parental allergy, asthma, or atopic dermatitis.

Oral itching and swellings/blisters were the most frequent subjective and objective symptoms, respectively (see Fig E2, A and B, in this article's Online Repository at www.jacionline.org). Involvement of more than 1 organ was seen in 14 of 35 participants. Upon challenge, 2 participants received adrenaline, but none had severe anaphylaxis requiring further observation or treatment.

Sensitization to all 3 fish-allergen extracts and parvalbumins was found in nearly all participants, whereas sensitization to enolases/aldolases was predominantly found in participants with objective symptoms (Fig 1, A-C). However, many participants with objective symptoms were not sensitized to the corresponding enolase/aldolase.

Nontolerant participants had higher sIgE levels to fish-allergen extracts and parvalbumins compared with partially tolerant participants, whereas no difference was seen for enolase and aldolase (see Table E3 in this article's Online Repository at www.jacionline.org). Specific IgE to extracts from cod and salmon discriminated best between nontolerant and partially tolerant groups. IgE level of more than 8.2 kU_A/L to cod extract or more than 5.0 kU_A/L to salmon extract identified 18 of 24 and 19 of 24 nontolerant participants, respectively, whereas below

TABLE I. Status of tolerance to cod, salmon, and/or mackerel, confirmed with DBPCFC/OFC

Status of tolerance	Objective allergic symptoms, n (%)	Any allergic symptoms, n (%)
Nontolerant	15 (43)	24 (68)
Partially tolerant	19 (54)	10 (29)
Tolerant*	1 (3)	1 (3)
Total	35 (100)	35 (100)

Nontolerant: Symptoms to cod, salmon, and mackerel. Partially tolerant: Symptoms to 1 or 2 of cod, salmon, and mackerel. Tolerant: No symptoms to cod, salmon, and mackerel.

*One participant with a convincing history of fish allergy turned out to be clinically tolerant to all 3 species. He was sIgE-positive to cod, salmon, and mackerel extracts (sIgE, 0.87, 0.79, and 0.15 kU_A/L, respectively), but not to any of the fish-allergen molecules, and had most likely outgrown his fish allergy.

TABLE II. Phenotypes of fish allergy in partially tolerant participants

Symptoms to	Objective allergic symptoms, n (%)	Any allergic symptoms, n (%)
Cod only	7 (20)	2 (6)
Salmon only	2 (6)	0
Mackerel only	0	0
Cod and salmon	6 (17)	3 (9)
Cod and mackerel	4 (11)	4 (11)
Salmon and mackerel	0	1 (3)
Total	19 (54)	10 (29)

these cutoff values, 8 of 10 and 9 of 10 partially tolerant participants were identified (see Fig E3 in this article's Online Repository at www.jacionline.org).

Finally, we estimated the eliciting dose predicted to provoke an allergic reaction in 10% of individuals (ED₁₀), on the basis of dose distribution curves (see Table E4 and Fig E4 in this article's Online Repository at www.jacionline.org). We found ED₁₀ to be 0.7 mg and 23.8 mg of cod protein for subjective and objective symptoms, respectively, in line with previously published data.⁸ Low number of participants with objective reactions to salmon and mackerel restricted us from producing reliable dose distribution curves but a comparison of lowest observed adverse effect levels (LOAELs) for objective symptoms to cod, salmon, and mackerel (see Fig E5, A-C, in this article's Online Repository at www.jacionline.org) shows more participants with low LOAELs for cod compared with salmon and mackerel.

Table E5 in this article's Online Repository at www.jacionline.org presents individual results from 35 participants with sIgE values and the results from DBPCFCs/OFCs.

This is the first DBPCFC trial comparing clinical and sIgE cross-reactivity to fish-allergen molecules with different fish species. More than half of all participants had objective tolerance, and around one-third had subjective tolerance to at least 1 fish species. By combining sIgE against conventional fish-allergen extracts and fish-allergen molecules, we could predict the outcome of challenge in most patients. Cod allergy was most prevalent and cod elicited more serious symptoms and had lower

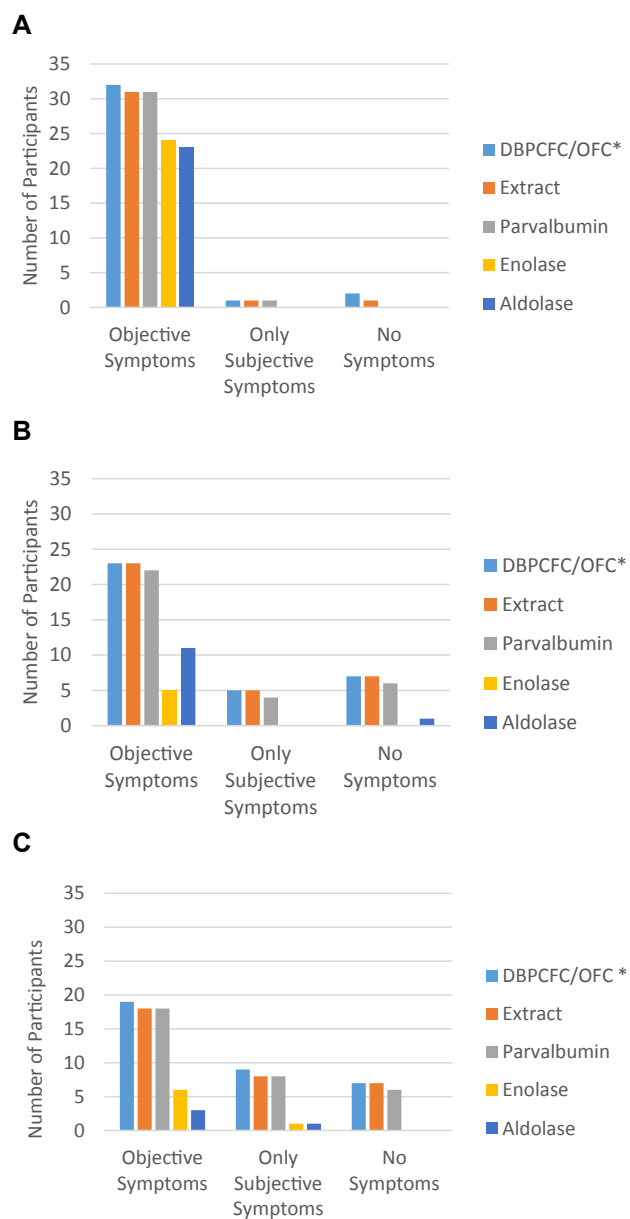


FIG 1. A, Sensitization to fish extracts and molecules in participants with objective ($n = 32$), only subjective ($n = 1$), and no symptoms ($n = 2$) to cod during DBPCFC/OFC. *DBPCFC/OFC = Food-challenge cod. Light blue bar, first group of bars: Number of participants with objective symptoms during cod challenge. Light blue bar, second group of bars: Number of participants with only subjective symptoms during cod challenge. Light blue bar, third group of bars: Number of participants with no symptoms during cod challenge. All other bars are number of participants sensitized ($sIgE > 0.1 \text{ kU}_A/\text{L}$) to cod using 4 different fish-allergen assays. **B,** Sensitization to fish extracts and molecules in participants with objective ($n = 23$), only subjective ($n = 5$), and no symptoms ($n = 7$) to salmon during DBPCFC/OFC. *DBPCFC/OFC = Food-challenge salmon. Light blue bar, first group of bars: Number of participants with objective symptoms during salmon challenge. Light blue bar, second group of bars: Number of participants with only subjective symptoms during salmon challenge. Light blue bar, third group of bars: Number of participants with no symptoms during salmon challenge. All other bars are number of participants sensitized ($sIgE > 0.1 \text{ kU}_A/\text{L}$) to salmon using 4 different fish-allergen assays. **C,** Sensitization to fish extracts and molecules in participants with objective ($n = 19$), only subjective ($n = 9$), and no symptoms ($n = 7$) to mackerel during DBPCFC/OFC. *DBPCFC/OFC = Food-challenge mackerel. Light blue bar, first group of bars: Number of participants with objective symptoms

ED₁₀/LOAELs, compared with salmon and mackerel. This may be due to local dietary traditions with high consumption of cod and cod being the primary sensitizer. Although unproven, differences in parvalbumin stability between species may theoretically play a role. The poor ability of fish-allergen extracts and parvalbumins to identify tolerance to specific fish species may be due to sIgE-cross-reactive parvalbumins.^{4,9} More sensitization to cod enolase/aldolase, compared with salmon and mackerel enolase/aldolase, may be due to less interspecies IgE cross-reactivity between enolases/aldolases and cod being the primary cause of sensitization.

Although allergen levels may vary in different allergen extract batches,⁹ patients with an obvious history of self-reported fish allergy and sIgE of more than 8.2 kU_A/L to cod extract or more than 5 kU_A/L to salmon extract may be advised to avoid all fish species. Mackerel extract is less reliable because median IgE to extract is lower than to parvalbumin (Table E3). Sensitization to enolase/aldolase most likely reflects true fish allergy, whereas nonsensitized patients may be allergic and must undergo a challenge. However, based on our results, an OFC protocol could be prepared for low-risk patients to test new fish species in small amounts without the need of a full DBPCFC. Our results indicate that sIgE to enolase/aldolase may have a role in diagnosing fish allergy when it comes to cod, salmon, and mackerel.

A limitation of this study is the low number of participants, attenuating the power to detect differences between groups. Second, children of lower age had higher dropout rates due to poor palatability of the test food, but none dropped out because of allergic reactions. We believe that the data represent good estimates of cross-reactivity and severity in the fish-allergic population but results may not be applicable to other regions. The main strength is the randomized, placebo-controlled design using standardized test food, followed by OFC. The basophil activation test will be included in a follow-up to further elaborate on the diagnostic differentiation between sensitization and true allergy.

In summary, more than half of the participants had no objective symptoms, and around one-third, no subjective symptoms to at least 1 fish species. Doses provoking allergic reactions are lower for cod than for salmon and mackerel. Patients with partial tolerance should be identified to avoid unnecessary food restrictions. A combination of clinical history and sIgE to fish-allergen extracts and molecules can significantly reduce the number of food challenges needed for specific diagnosis of fish allergy.

We are thankful to all the participants and their parents who spent up to 7 days to participate, many of them eating test food they did not like and gave them allergic reactions. We greatly appreciate the professional work of the staff at the Department of Research, University Hospital of North Norway for performing all the DBPCFCs and OFCs. Without their enthusiastic contribution, this study would not have been possible.

during mackerel challenge. Light blue bar, second group of bars: Number of participants with only subjective symptoms during mackerel challenge. Light blue bar, third group of bars: Number of participants with no symptoms during mackerel challenge. All other bars are number of participants sensitized ($sIgE > 0.1 \text{ kU}_A/\text{L}$) to mackerel using 4 different fish-allergen assays.

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The following sources funded this study: Northern Norway Regional Health Authority; University of Tromsø; University Hospital of North Norway; the Norwegian Asthma and Allergy Association, Research fund; Odd Berg Group, Medical Research fund; the Norwegian Allergology and Immunopathology Association, Research fund; Klosterstiftelsen, Research fund for asthma and allergy in children; the Ministry of Higher Education and Research (MESR), Luxembourg; and Thermo Fischer Scientific A/B (supplied ImmunoCAP tests free of charge). None of the funding sources was involved in study design, collection, analysis, or interpretation of data, in writing the report, or the decision to submit the article for publication.

Disclosure of potential conflict of interest: M. Sørensen receives grant support from the Northern Norway Regional Health Authority; the Department of Clinical Medicine UiT the Arctic University of Norway; University Hospital of North Norway; the Norwegian Asthma and Allergy Association, research fund; Odd Berg Group, Medical Research fund; the Norwegian Allergology and Immunopathology Association, research fund; and Thermo Fischer Scientific A/B; supplied ImmunoCAP tests free of charge; and received payment for lectures from Thermo Fisher Scientific. A. Kuehn

receives grant support from the Luxembourg Ministry of Higher Education and Research. C. A. Costello receives travel support from Short Term Scientific Mission Reference COST Action FA1402. M. Ollert receives grant support from the Luxembourg Ministry of Higher Education and Research and payments for lectures from ThermoFisher Scientific. M. Wickman serves as a consultant for ThermoFisher Scientific, MEDA, and Stallergenes and received payments for lectures from ThermoFisher Scientific. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Mourad AA, Bahna SL. Fish-allergic patients may be able to eat fish. *Expert Rev Clin Immunol* 2015;11:419-30.
2. Helbling A, Haydel R Jr, McCants ML, Musmand JJ, El-Dahr J, Lehrer SB. Fish allergy: is cross-reactivity among fish species relevant? Double-blind placebo-controlled food challenge studies of fish allergic adults. *Ann Allergy Asthma Immunol* 1999;83:517-23.
3. Bernhisel-Broadbent J, Scanlon SM, Sampson HA. Fish hypersensitivity, I: in vitro and oral challenge results in fish-allergic patients. *J Allergy Clin Immunol* 1992; 89:730-7.
4. Van Do T, Elsayed S, Florvaag E, Hordvik I, Endresen C. Allergy to fish parvalbumins: studies on the cross-reactivity of allergens from 9 commonly consumed fish. *J Allergy Clin Immunol* 2005;116:1314-20.
5. Sharp MF, Lopata AL. Fish allergy: in review. *Clinic Rev Allerg Immunol* 2014; 46:258-71.
6. Aas K, Elsayed SM. Characterization of a major allergen (cod): effect of enzymic hydrolysis on the allergenic activity. *J Allergy* 1969;44:333-43.
7. Kuehn A, Hilger C, Lehnert-Weber C, Codreanu-Morel F, Morisset M, Metz-Favre C, et al. Identification of enolases and aldolases as important fish allergens in cod, salmon and tuna: component resolved diagnosis using parvalbumin and the new allergens. *Clin Exp Allergy* 2013;43:811-22.
8. Ballmer-Weber BK, Fernandez-Rivas M, Beyer K, Defernez M, Sperrin M, Mackie AR, et al. How much is too much? Threshold dose distributions for 5 food allergens. *J Allergy Clin Immunol* 2015;135:964-71.
9. Kuehn A, Scheuermann T, Hilger C, Hentges F. Important variations in parvalbumin content in common fish species: a factor possibly contributing to variable allergenicity. *Int Arch Allergy Immunol* 2010;153:359-66.

<http://dx.doi.org/10.1016/j.jaci.2017.03.043>

Table E1, Online Repository. Background characteristics and allergy phenotype of participants, dropouts and eligible patients not included.

	Included			Not included	
	Completed N=35	Dropouts N=9	P†	N=29	P‡
Mean age (SD)	11.6 (3.0)	6.7 (1.4)	<0.001	11.6 (3.9)	0.256
Sex (Boys)	22 (63%)	7 (78%)	0.695	20 (69%)	0.619
Asthma*	21 (60%)	4 (44%)	0.467	15 (52%)	0.811
Atopic dermatitis*	24 (69%)	9 (100 %)	0.085	22 (76%)	1.000
Allergic rhinitis*	23 (66%)	6 (67%)	1.000	20 (69%)	1.00
Median (IQR) sIgE Cod	10.8 (5.5-25.9)	38.6 (10.3-115.1)	0.089	12.7 (2.4-53.3)	0.727
Median (IQR) sIgE Salmon	8.4 (2.6-19.8)	40.5 (5.9-55.0)	0.190	15.3 (5.5-74.1)	0.413
Parental allergic disease	28 (80%)	7 (78%)	1.000	18/22 (82%)	1.000
Food allergy, other than fish	31 (89%)	8 (89%)	1.000	27 (93%)	0.160

Included = Included in the study (n=44), Not included = Not consenting to participate (n=26) or did not meet inclusion criteria (n=3). Dropouts = dropouts from originally included participants. *Symptoms recorded at the Paediatric outpatient clinic, University Hospital of North Norway. SD = Standard Deviation. IQR= Interquartile range. P†; comparison between completed and dropouts. P‡; comparison between all included and not included (comparison of sIgE to cod and salmon extracts by Mann Whitney U Test).

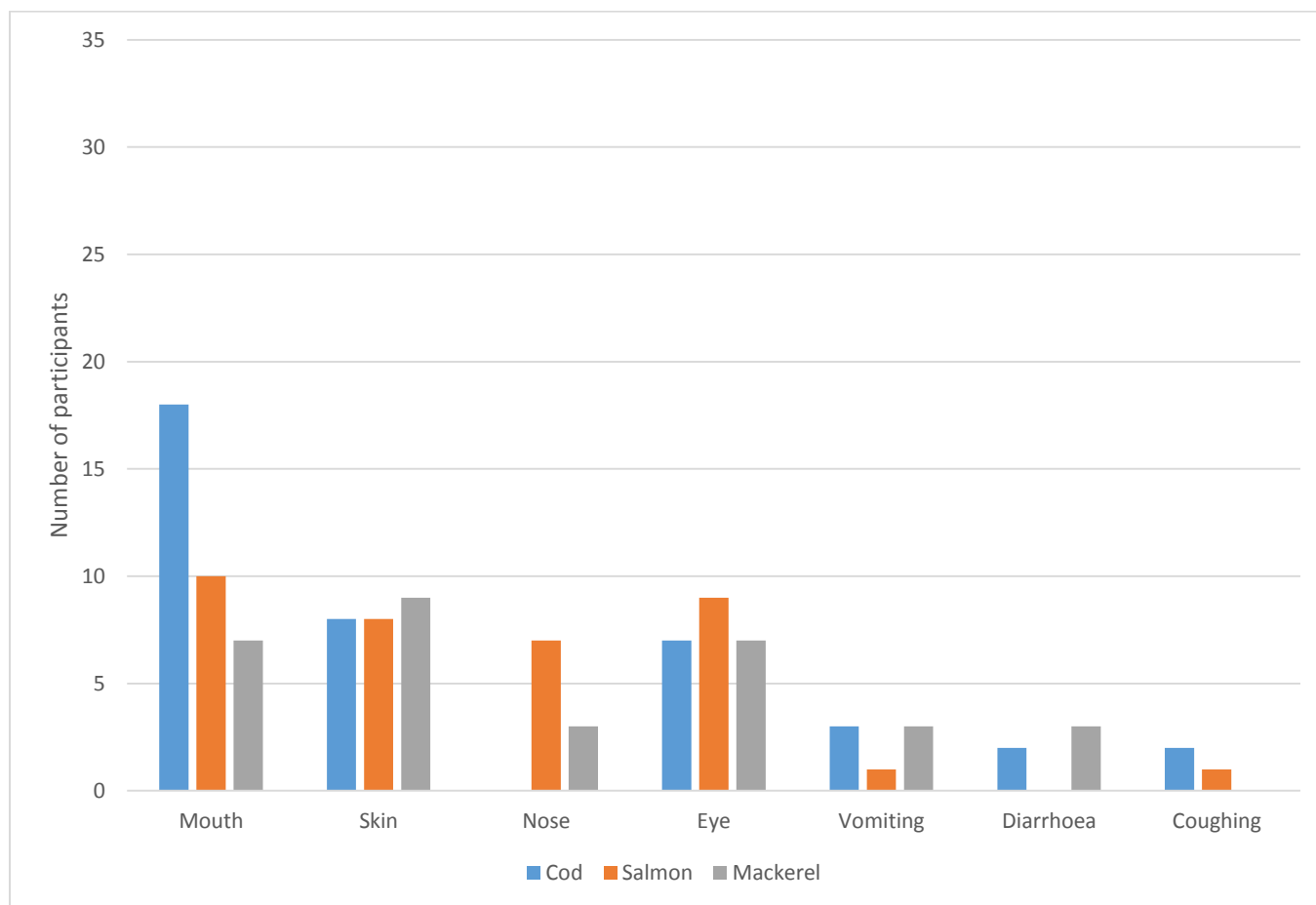
Table E2, Online Repository. Symptoms upon DBPCFC/OFC in participants with placebo reactions

Pat Nr.	Fish Spec.	Fish challenge								Clinical reactivity Non-tolerant/Partially tolerant	
		DBPCFC doses				OFC doses				Any symptoms	Objective symptoms
		3 µg	600 µg	12.5mg	120 mg	1 g	2 g	6 g	12 g		
22	Cod		Itchy Mouth VAS 2	Itchy Mouth VAS 6	Itchy Mouth VAS 7 Lip Blisters					NT	NT
	Salmon			Erytema Face	Erytema Face Itchy Mouth VAS 3	Erytema Face Urticaria Itchy Mouth VAS 5					
	Mackerel			Erytema Face Urticaria Itchy Mouth VAS 1	Erytema Face Urticaria Itchy Mouth VAS 6	Erytema Face Urticaria Lip Blister Itchy Mouth VAS 2					
	Placebo			Itchy Mouth VAS 3	Itchy Mouth VAS 6	Itchy Mouth VAS 6					
23	Cod			Itchy Mouth VAS 6	Itchy Mouth VAS 8 Abd. Pain VAS 10	Skin Erytema Itchy Mouth VAS 3 Abd. Pain VAS 10				NT	PT Tolerant to mackerel
	Salmon		Abd. Pain VAS 7	Itchy Skin VAS 10 Urticaria Eye lid edema							
	Mackerel						Itchy Mouth VAS 6				
	Placebo					Abdominal Pain VAS 6 *					
31	Cod			Itchy Mouth VAS 7 Throat pain VAS 5	Itchy Mouth VAS 7 Nausea VAS 7 Throat pain VAS 8 Lip Blisters					NT	NT
	Salmon				Itchy Mouth VAS 7	Itchy Mouth VAS 8	Itchy Mouth VAS 9 Itchy Eye Sneeze x 6				
	Mackerel					Itchy Mouth VAS 6	Itchy Mouth VAS 9 Itchy Eyes				

							Red Eyes				
	Placebo		Itchy Mouth VAS 5	Itchy Mouth VAS 5							
32	Cod			Itchy Mouth VAS 4	Itchy Mouth VAS 8 Red spots on swollen tounge					PT Tolerant to salmon and mackerel	PT Tolerant to salmon and mackerel
	Salmon										
	Mackerel										
	Placebo	Itchy Mouth VAS 1	Itchy Mouth VAS 1	Itchy Mouth VAS 1							
33	Cod		Itchy Mouth VAS 2	Itchy Mouth VAS 1	Itchy Mouth VAS 2 Chest pain VAS 5 Itchy skin, erytema Tired/Fatigue					NT	NT
	Salmon						Itchy skin VAS 4 Throat tightness VAS 6 Red eyes, Urticaria				
	Mackerel				Itchy Mouth VAS 2	Itchy Mouth VAS 3 Itchy Skin VAS 6 Skin eryteme					
	Placebo			Itchy Mouth VAS 2	Itchy Mouth VAS 2	Itchy Mouth VAS 2					

NT = Non-Tolerant. PT = Partially tolerant*The participant thought he experienced abdominal pain because he was satisfied after the last dose of DBPCFC. The pain was relieved after 10 minutes. VAS = Visual Analog Scale (0-10)

Figure E2a, Online Repository. Number of participants (N=35) with objective symptoms to cod, salmon and mackerel during DBPCFC/OFC.



Mouth: swellings/blisters in the mouth or on the lips. Skin: Erythema, urticaria or angioedema of the skin. Nose: Sneeze or itchy, runny, blocked nose. Eye: Itchy, red or watery eyes. No participants had stridor, wheeze, tachycardia, arrhythmia, syncope, seizures or incontinence.

Fig E2b, Online Repository. Number of participants (N=35) with subjective symptoms to cod, salmon and mackerel (DBPCFC/OFC)

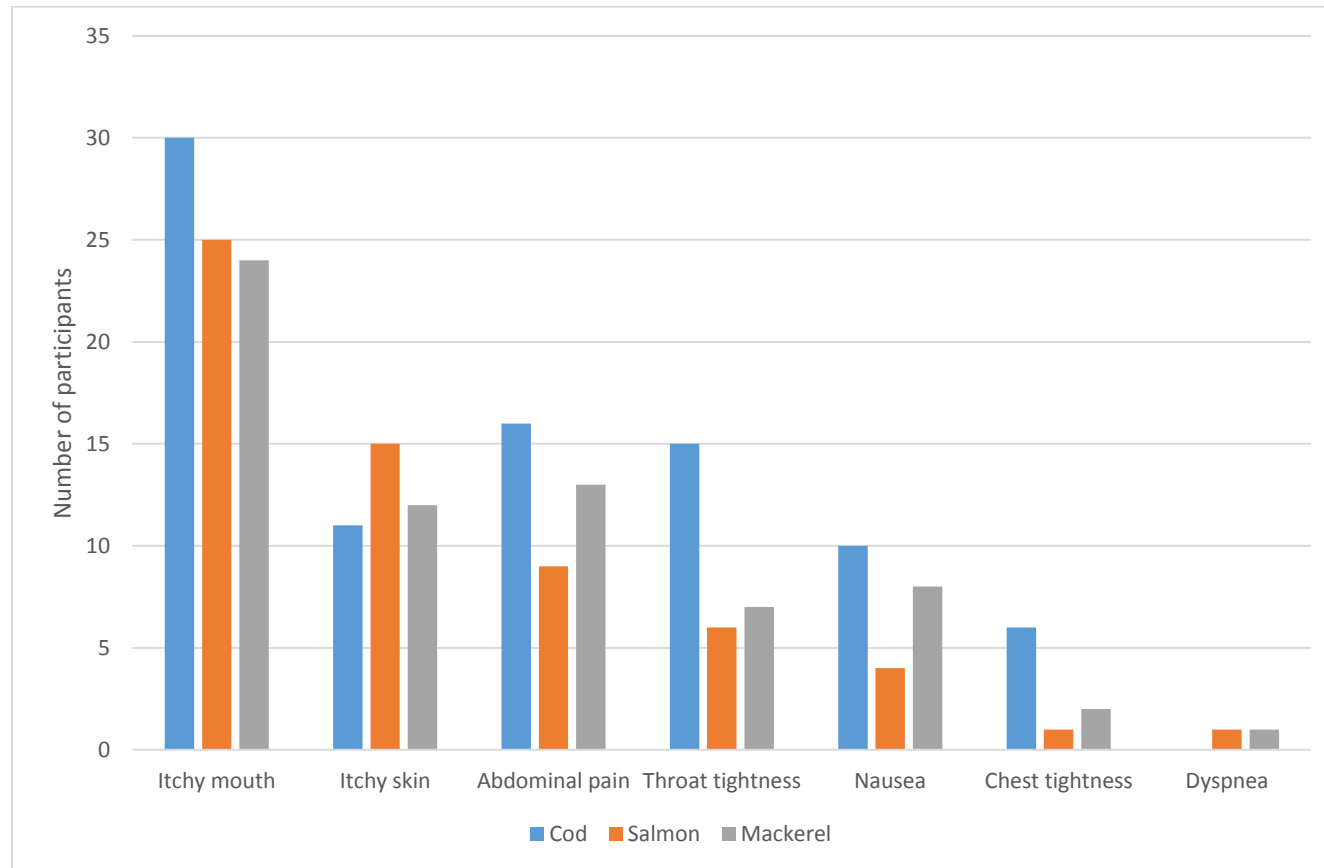


Figure E3, Online Repository. ROC curves for discrimination between partially tolerant and non-tolerant participants, subjective symptoms.

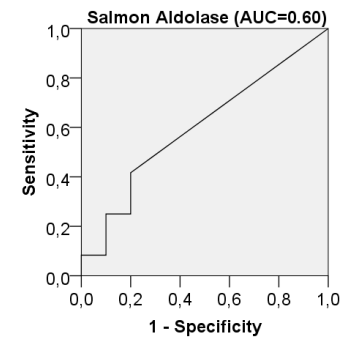
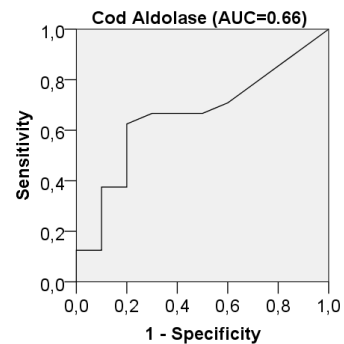
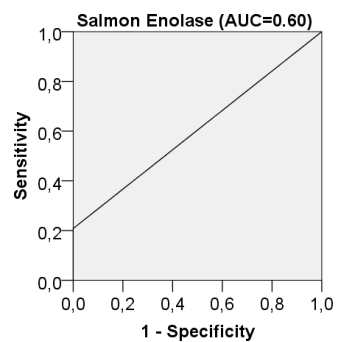
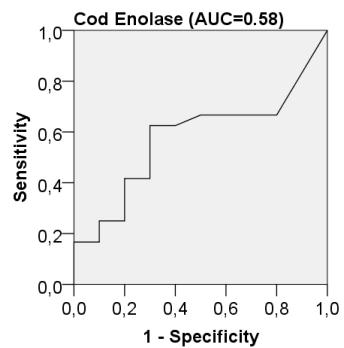
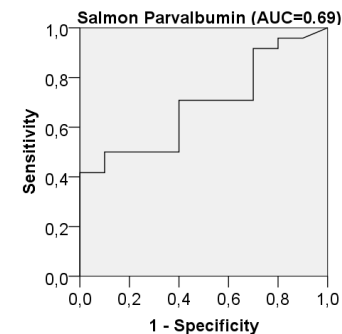
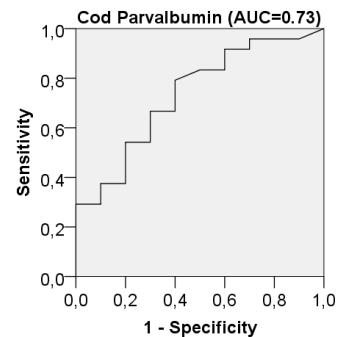
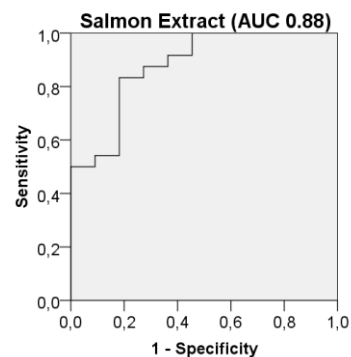
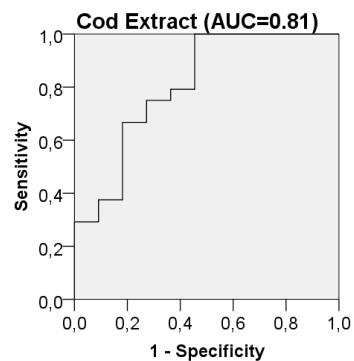
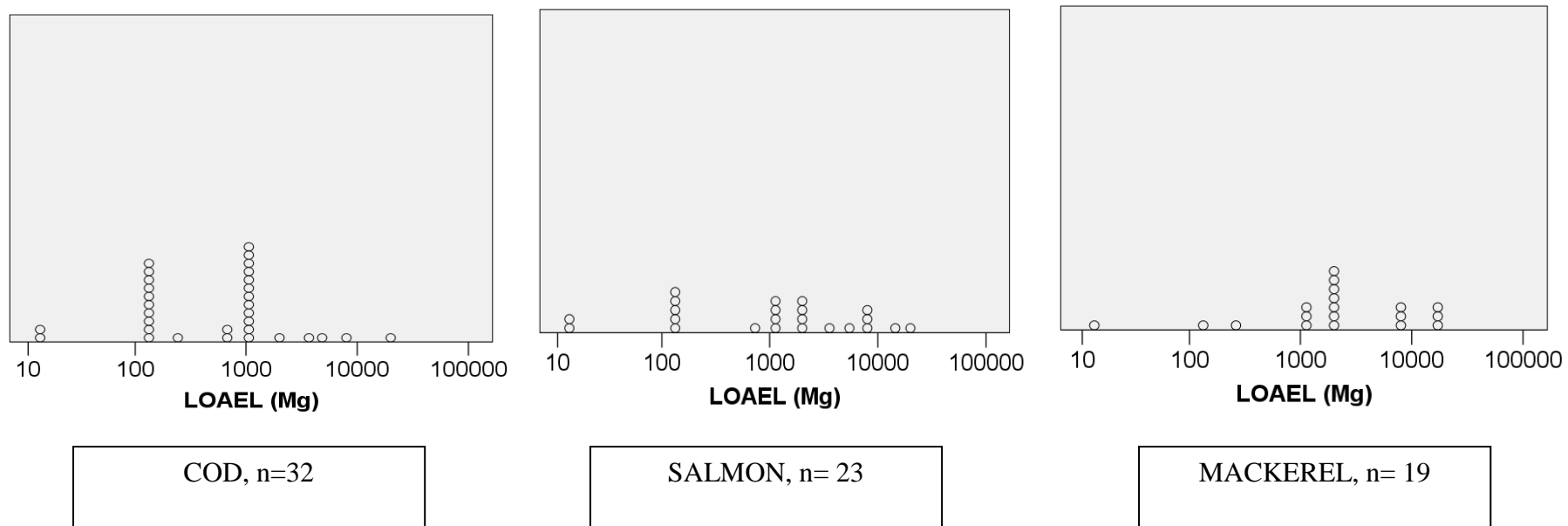


Figure E5, Online Repository. Lowest Observed Adverse Effect Level (LOAEL) for objective symptoms to cod, salmon and mackerel during DBPCFC/OFC



Each circle represents one participant.

Table E5, Online Repository. Subjective, objective and anaphylactic symptoms at each dose during DBPCFC/OFC and IgE values for each participant.

Pat. Nr.	Fish Spec.	Fish challenge								sIgE to fish-allergens				Clinical Reactivity	
		DBPCFC doses				OFC doses				Extr.	Parv.	Enol.	Aldo.	Non-tolerant /Partially tolerant	
		3µg	600 µg	12.5mg	120 mg	1 g	2 g	6 g	12 g					Any symptoms	Objective symptoms
1	Cod				S	S	S		X	10.70	4.90	.10	.10	PT Tolerant to Mackerel	PT Tolerant to Mackerel and Cod
	Sal				SO	SOX				14.50	11.70	.10	6.70		
	Mac								X	4.70	19.00	.10	.10		
	Pla					X									
2	Cod					SOAX				117.00	121.70	61.50	63.10	NT	NT
	Sal					SO	S	SOA	SOAX E	144.00	30.30	9.50	18.30		
	Mac						S	S	SOAX	52.10	49.20	1.10	.50		
	Pla					X									
3	Cod				S	SOX				5.98	5.50	.10	1.00	NT	NT
	Sal				S	SO	SOX			6.69	6.60	.10	.80		
	Mac					SO	S	SOX		1.67	8.10	.10	.10		
	Pla					X									
4	Cod		S	S	SO	SOX				288.00	153.00	5.40	4.90	NT	NT
	Sal				S	S	SOX			254.00	205.00	7.10	46.10		
	Mac				S	S	S	SO	SOX	41.00	200.00	.10	.10		
	Pla					X									
5	Cod				SOX					28.30	20.60	3.50	4.10	PT Tolerant to salmon	PT Tolerant to salmon
	Sal							X		2.64	13.50	.10	.10		
	Mac					S		SOX		2.34	11.20	.10	.10		
	Pla					X									
6	Cod				S	SOAX				25.90	16.80	.10	.10	NT	NT
	Sal				S	SOAX				20.10	14.10	.10	.10		
	Mac						S	S	SOAX	7.25	13.10	.10	.10		
	Pla					X									
7	Cod						S	S	SOAX	6.38	5.30	1.10	1.50	PT Tolerant to salmon and mackerel	PT Tolerant to salmon and mackerel
	Sal								X	4.65	4.70	.10	.10		
	Mac								X	1.84	3.40	.10	.10		
	Pla					X									
8	Cod			S	SOX					3.38	2.70	.50	.80	NT	NT

	Sal					SO	SO	SOX		3.24	3.00	.10	.40		
	Mac							SOX		.89	2.50	.10	.10		
	Pla					X									
9	Cod			S	S	SOX				15.30	20.80	.10	.10	NT	PT
	Sal				SO	SO	SOX			27.30	30.10	.10	2.10		Tolerant to mackerel
	Mac				S	S	S	S	SX	5.53	16.20	.10	.10		
	Pla					X									
10	Cod					SO		SOX		.07	11.30	1.50	.10	PT	PT
	Sal							X		.92	10.90	.10	1.40	Tolerant to salmon	Tolerant to salmon
	Mac					SO		SOX		.04	6.80	.30	.40		
	Pla					X									
11	Cod					S	S	SO	SOX	6.40	5.60	1.30	1.80	NT	NT
	Sal				SO	SO	SOAX			7.75	5.40	.10	1.60		
	Mac						SO	SO	SOX	2.13	7.50	.10	.10		
	Pla					X									
12	Cod				S	SO	SO	SOAX		1.46	1.50	.50	.60	PT	PT
	Sal			S	S	S	S	S	SX	4.20	3.20	.10	.10	Tolerant to mackerel	Tolerant to salmon and mackerel
	Mac								X	.39	2.10	.10	.10		
	Pla					X									
13	Cod			SO	SOAX					70.00	108.0	2.70	3.90	NT	NT
	Sal			S	S	SOAX				45.90	69.70	.10	.10		
	Mac			S	SOAX		SOAX			9.69	67.70	.10	.10		
	Pla					X									
14	Cod								X	.87	.10	.10	.10	Tolerant to all three species	Tolerant to all three species
	Sal								X	.79	.10	.10	.10		
	Mac								X	.15	.10	.10	.10		
	Pla					X									
15	Cod				SO	SOX				3.20	4.80	.10	.30	NT	PT
	Sal				O	O	SO	SO	SOX	1.80	2.50	.10	.10		Tolerant to mackerel
	Mac							S	SX	.67	1.90	.10	.10		
	Pla					X									
16	Cod						SO	SOX		1.57	.50	.30	.30	PT	PT
	Sal							SO	SOX	.23	.70	.10	.10	Tolerant to mackerel	Tolerant to mackerel
	Mac							X		.12	.60	.10	.10		
	Pla					X									
17	Cod			SO	SOAX					14.00	10.80	.90	1.30	NT	NT
	Sal				S	S	SOX			10.80	12.40	.10	2.10		

	Mac				S	SO	SO	SOX		5.49	12.70	.10	.10		
	Pla					X									
18	Cod				S	SOAX				20.90	27.60	.30	.10	PT Tolerant to salmon	PT Tolerant to salmon and mackerel
	Sal							X		13.10	10.70	.10	.10		
	Mac							SX		2.58	8.70	.10	.10		
	Pla					X									
19	Cod				S	S	SOAX			8.78	.10	.90	1.70	NT	PT Tolerant to salmon
	Sal				S	S	S	S	X	8.37	.10	.10	.10		
	Mac					S	SOAX			2.37	.10	.40	.10		
	Pla					X									
20	Cod				S	SO	SOX			46.30	44.60	70.00	83.00	NT	PT Tolerant to mackerel
	Sal				SO		SOX			88.60	17.00	.90	.10		
	Mac						SX			13.30	23.70	1.10	1.00		
	Pla					X									
21	Cod				SO		SO	SOX		1.47	.70	.30	.40	PT Tolerant to salmon and mackerel	PT Tolerant to salmon and mackerel
	Sal							X		.48	1.40	.10	.10		
	Mac							X		.28	1.30	.10	.10		
	Pla					X									
22	Cod		S	S	SOX					5.47	5.30	.10	1.30	NT	NT
	Sal			O	SO	SO		SO	SOX	4.37	4.90	.10	.80		
	Mac			SO	SO	SOX				1.37	5.60	.10	.10		
	Pla			S	S	SX									
23	Cod				S	S	SOX			18.90	11.60	.90	1.10	NT	PT Tolerant to mackerel
	Sal		S		SOX					11.70	8.90	.10	.10		
	Mac						SX			5.70	9.20	.10	.10		
	Pla						SX								
24	Cod				S	SOAX				10.10	10.00	.80	1.40	NT	PT Tolerant to salmon and mackerel
	Sal						S	S	SX	5.36	2.60	.10	.10		
	Mac				S	S	S	S	SX	2.12	2.50	.10	.10		
	Pla					X									
25	Cod								X	.01	.10	.10	.10	PT Tolerant to cod	PT Tolerant to cod and mackerel
	Sal					S			SOX	.10	.10	.10	.10		
	Mac						S	S	SX	.01	.10	.10	.10		
	Pla					X									
26	Cod						S	SOX		31.60	32.50	6.20	.10	NT	PT Tolerant to mackerel
	Sal						S	SOX		25.80	19.80	.10	.10		
	Mac						SX			12.20	15.90	.10	.10		

	Pla														
27	Cod					S	S	SOX		22.00	25.40	.10	.10	NT	NT
	Sal		S	S	S	S	S	SOX		13.50	27.90	.40	.70		
	Mac							SOX		3.88	27.10	.10	.10		
	Pla					X									
28	Cod				S	SO	SO	SOX		49.30	41.10	.10	.10	NT	NT
	Sal						S	SO	SOX	19.80	22.70	.10	.10		
	Mac						S	SO	SOX	5.33	27.80	.10	.10		
	Pla					X									
29	Cod			S	S	SOAX				14.70	8.20	1.20	1.60	NT	NT
	Sal						S	SOX		7.69	2.30	.10	.10		
	Mac						S	S	SOAX	7.02	2.30	.10	.10		
	Pla					X									
30	Cod			S	S	SOAX				5.89	6.50	1.30	1.70	NT	PT Tolerant to salmon
	Sal							SX		2.08	2.30	.10	.10		
	Mac						S	SOA	SOAX	.35	3.30	.50	.10		
	Pla					X									
31	Cod			S	SOX					19.50	13.40	1.60	1.80	NT	NT
	Sal				S	S		SOX		16.10	10.30	1.30	3.70		
	Mac					S		SOX		9.37	16.00	.30	.20		
	Pla	S		SX											
32	Cod			S	SOX					7.77	8.00	.60	.70	PT Tolerant to salmon and mackerel	PT Tolerant to salmon and mackerel
	Sal							X		.89	4.20	.10	.10		
	Mac							X		.71	2.70	.10	.10		
	Pla	S	S	S				X							
33	Cod		S	S	SOAX					0.92	.80	.10	.10	NT	NT
	Sal							SOAX		0.23	1.00	.10	.10		
	Mac				S	SOX				0.39	.80	.10	.10		
	Pla			S	S	SX									
34	Cod			S	SO	SOX				37.50	38.40	.90	.70	NT	NT
	Sal				S	S	S	SO	SOX	23.60	35.60	.10	.10		
	Mac				S	S		SOAXE		9.76	58.50	.40	.10		
	Pla					X									
35	Cod			S	S	SOX				13.60	15.40	1.40	.10	NT	
	Sal				S		S	S	SX	12.70	12.10	.10	.10		
	Mac				S		S	S	SX	2.94	13.40	.10	.10		
	Pla					X									

DBPCFC = Double Blind Placebo Controlled Food Challenge. OFC = Open Food Challenge. Sal = salmon, Mac = mackerel, Pla = placebo, Extr = extract, Parv. = parvalbumin, Enol. = enolase, Aldo. = aldolase, S = subjective allergic reaction, O = objective allergic reaction, A = anaphylactic reaction, X = challenge stopped during/after this dose. E = Epinephrine auto injector 0.3 mg intramuscular injection. Participants are numbered 1-35. IgE is measured in kU_A/L. NT = Non-tolerant.